OIPE JC86 MAR 11 2003 PATENT & TRADEMARK OFFICE

AMENDMENT

IN THE SPECIFICATION:

Paragraph beginning at line 8 of page 7 has been amended as follows:

Figures 6A-6B show ~~6 shows~~ the binding titer and specificity of HuM195-gelonin immunotoxin on cell lines. HL60, U937 or Molt4 cells at a concentration of 1.5 x 10^6 cells/ml were incubated on ice for 1 hour with either HuM195 or MuM195-gelonin at a final concentration range of 0.08 to 10 µg/ml. Mean peak fluorescence intensity (y-axis) versus mAb or immunotoxin (IT) concentration (x-axis) was measured using an EPICS Profile flow cytometer. **Figure 6A** ~~Panel A~~ shows HL60 binding by HuM195-gelonin immunotoxin (•) or by HuM195 alone (o). **Figure 6B** ~~Panel B~~ shows U937 binding by HuM195-gelonin immunotoxin (•) or by HuM195 alone (o); Molt4 binding by HuM195-gelonin immunotoxin (filled square) or by HuM195 alone(Δ).

Paragraph beginning at line 6 of page 8 has been amended as follows:

Figures 9A-9B show ~~9 shows~~ the cytotoxicity and inhibition of protein synthesis in HL60 or RAJI cells by recombinant gelonin (rGel), HuM195 or the HuM195-rGel immunotoxin. **Figure 9A** ~~Panel A~~ shows the inhibition of protein synthesis in HL60 or RAJI cells by rGel, HuM195 and HuM195-rGel. HL60 or RAJI cells at a final concentration of 10^5 cells/ml were incubated 3 days at 37°C in the presence of HuM195-rGel, rGel or HuM195. Levels of protein

synthesis were determined by 5 hour incorporation of tritiated leucine into trichloroacetic acid precipitable protein. The treatment is shown in parenthesis. **Figure 9B** ~~Panel B~~ shows cell viability determined by typan blue exclusion. HL60 or RAJI cells at a final concentration of 10^5 cells/ml were incubated 3 days at 37°C in the presence of HuM15-rGel. Typan blue was added and live and dead cells were counted under the microscope.

C2 Cont'd

Paragraph beginning at line 15 of page 9 has been amended as follows:

Figures 12A-12C show ~~1 2 shows~~ the enhanced effect of the immunotoxin when combined with cryopreservation. The AML cell lines OCI/AML3 (CD33neg), NB4 (CD33pos), HL60 (CD33pos) or patient samples, all CD33 positive, were plated after freeze/thaw alone (A), 1 nM immunotoxin for 24 hours (B), or immunotoxin followed by freeze/thaw (C). Mean colony number was determined from 4 replicate wells and is expressed as percent of control.

C3

Paragraph beginning at line 25 of page 10 has been amended as follows:

Figures 17A-17B show ~~1 7 shows~~ the treatment of human leukemia cells *in vivo* by HuM195-rGel immunotoxin. Mice were i.p. transplanted with 10^7 HL60 human leukemia cells. **Figure 17A** ~~Panel A~~ shows that at the tenth day, mice were treated by three injections of 100 nM: HuM195-rGel (four mice); rGel (four mice); HuM195 mixed with rGel (five mice); and control saline (five mice). At the time indicated by the x-axis, tumor surface area was measured. One of five mice in the control group and one of the five

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mice in the HuM195-rGel (mixed but unconjugated) died in the sixth week. **Figure 17B** ~~Panel B~~ shows that at the 14th or 28th days, mice were treated with 6 injections of 100 nM: HuM195-rGel immunotoxin (four mice at the 14th day; four mice at the 28th day); control saline (five mice). At times indicated by the x-axis, tumor surface area was measured.

C4 contd

Paragraph beginning at line 17 of page 28 has been amended as follows:

With reference to Figures 6A-6B, ~~6,~~ the binding of humanized M195 (HuM195)-immunotoxin to HL60, U937 or MOLT4 cells were examined. HuM195 and HuM195-immunotoxin were added to HL60 cells (Figure 6A, ~~panel A~~) or U937 or MOLT4 cell lines (Figure 6B, ~~panel B~~). Figures 6A-6B illustrate ~~6 illustrates~~ that the humanized M195-gelonin immunotoxin is capable of binding specifically to target cells. Using indirect flow cytometry, the humanized M195-gelonin immunotoxin showed more specific binding to CD33 positive cell lines (HL60 and U937) compared to the humanized M195 antibody alone. It did not bind to the CD33 negative cell line (MOLT4).

C5

Paragraph beginning at line 6 of page 35 has been amended as follows:

Treatment of AML with high dose chemotherapy followed by transplantation of cryopreserved autologous bone marrow is frequently unsuccessful due to the presence of leukemic blast progenitors in the bone marrow autograft. Cryopreservation itself has some antileukemic effects, to which other purging modalities

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have been added. To determine whether cryopreservation would enhance the effect of HuM195-rGel against CD33 positive AML blasts, cells from 12 patient samples were resuspended in freezing medium (10% DMSO; 50% FEBS) immediately following a 24 hour exposure to HuM195-rGel (1 nM), frozen at -70°C for a minimum of 24 hours, quickly thawed by immersion in a 37°C water bath, washed several times and plated on methylcellulose to determine clonogenic cell recovery. The data shown in Figures 12A12C ~~12~~ indicate that there is a greater than additive effect against all but one patient sample.

C6 cont'd

Paragraph beginning at line 20 of page 37 has been amended as follows:

The leukemic cell growth in the subcutaneous space and peritoneum of nude mice was substantially reduced by HuM195-rGel. At 10 days after transplantation of HL60 cells into the peritoneum of nude mice, tumors of about 2 mm^3 in size were present in the subcutaneous space (Figures 17A-17B ~~17~~). After three injections of HuM195-rGel at a dose of 36 µg per mouse beginning at 10 days, two out of four mice did not develop tumors for up to 5 months after transplantation. Tumors grew slowly in the other two immunotoxin-treated mice. Control groups of mice (treatment with saline alone, gelonin alone or HuM195 mixed with rGel at the same final concentrations) did not show significant tumor inhibition or any cures.

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